

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification<sup>6</sup>:</b> <b>A61K 31/44, 31/425, 31/19</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 98/29120</b> <b>(43) International Publication Date:</b> 9 July 1998 (09.07.98)
<b>(21) International Application Number:</b> PCT/US97/24187 <b>(22) International Filing Date:</b> 29 December 1997 (29.12.97) <b>(30) Priority Data:</b> 60/034,133 31 December 1996 (31.12.96) US <b>(71) Applicant (for all designated States except US):</b> THE SALK INSTITUTE FOR BIOLOGICAL STUDIES [US/US]; 10010 North Torrey Pines Road, San Diego, CA 92037 (US). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> EVANS, Ronald, M. [US/US]; 1471 Cottontail Lane, La Jolla, CA 92037 (US). TOTONAZ, Peter [US/US]; 3465 Lebon Drive #1727, San Diego, CA 92122 (US). SPIEGELMAN, Bruce, M. [US/US]; 271 Waban Avenue, Waban, MA 02168 (US). FORMAN, Barry, M. [US/US]; Apartment 299, 8568 Via La Jolla, La Jolla, CA 92037 (US). <b>(74) Agent:</b> REITER, Stephen, E.; Gray Cary Ware & Freidenrich, Suite 1600, 4365 Executive Drive, San Diego, CA 92121 (US).	<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>	
<b>(54) Title:</b> TREATMENT OF LIPOSARCOMAS USING A COMBINATION OF THIAZOLIDINEDIONES AND RETINOID X RECEPTOR SELECTIVE AGONISTS		
<b>(57) Abstract</b>  In accordance with the present invention, it has been discovered that PPAR $\gamma$ is expressed consistently in each of the major histologic types of human liposarcoma. It has further been discovered that maximal activation of PPAR $\gamma$ with exogenous ligand (a thiazolidinedione or derivative thereof) promotes terminal differentiation of primary human liposarcoma cells. It has still further been discovered that RXR-specific ligands are also potent adipogenic agents in cells expressing the PPAR $\gamma$ /RXR $\alpha$ heterodimer, and that simultaneous treatment of liposarcoma cells with a thiazolidinedionyl moiety (a PPAR $\gamma$ -selective class of compounds) and an RXR-specific ligand results in an additive stimulation of differentiation. Accordingly, according to the invention, there have been identified compositions which are useful for the treatment of liposarcomas.		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AI	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

Treatment of liposarcomas using a combination of Thiazolidinediones and retinoid X receptor selective agonists.

#### FIELD OF THE INVENTION

The present invention relates to methods for the treatment of liposarcomas. In another aspect, the present invention relates to compounds and compositions which are  
5 useful for carrying out the above-referenced methods.

#### BACKGROUND OF THE INVENTION

Liposarcoma is the most common soft tissue malignancy in adults, accounting for at least 20% of all sarcomas in this age group. Multiple histologic subtypes  
10 of liposarcoma are recognized, including well differentiated, de-differentiated, myxoid, round cell and pleimorphic. The histologic subtype is predictive of both the clinical course of the disease and the ultimate prognosis.

15 Localized disease is treated primarily with surgery, often in combination with radiotherapy. Metastatic liposarcoma is associated with an extremely poor prognosis, with average five year survival ranging from 70% to 25%, depending on the type of tumor. Unfortunately,  
20 conventional chemotherapy for metastatic liposarcoma leads to complete response in only about 10% of cases. Thus, for most patients, conventional chemotherapy is largely palliative.

Induction of terminal differentiation represents a promising alternative to conventional chemotherapy for certain malignancies. For example, the retinoic acid receptor alpha ( $RAR\alpha$ ), which plays an important role in the differentiation and malignant transformation of cells of myelocytic lineage, has been used as a target for intervention in acute promyelocytic leukemia. Indeed, differentiation therapy with all-trans retinoic acid has become the standard of care for this disease. In view of this success, it has been speculated that nuclear receptors that regulate growth and differentiation of other cell types may also represent potential targets for differentiation therapy.

Recent years have seen important advances in the understanding of the molecular basis of adipocyte differentiation. Central to this process is the induction of the adipocyte-selective nuclear receptor, peroxisome proliferator-activated receptor gamma ( $PPAR\gamma$ ). This receptor and its heterodimeric partner, the retinoid X receptor alpha ( $RXR\alpha$ ), form a DNA binding complex that regulates transcription of adipocyte-specific genes. Expression and activation of  $PPAR\gamma$  in fibroblastic cells triggers the adipocyte gene expression cascade and leads to development of the adipose phenotype.

Accordingly, the development of effective, non-invasive methods for treating liposarcomas would represent a significant advancement in the therapeutic arts.

#### BRIEF DESCRIPTION OF THE INVENTION

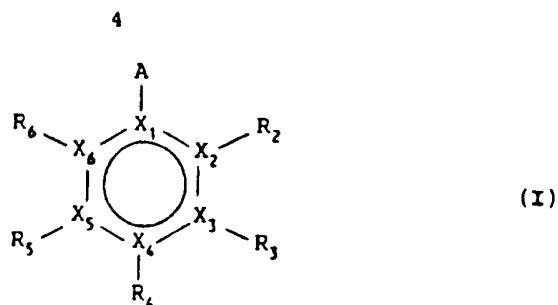
In accordance with the present invention, we have discovered that  $PPAR\gamma$  is expressed consistently in each of the major histologic types of human liposarcoma. It has further been discovered that maximal activation of  $PPAR\gamma$  with exogenous ligand (a thiazolidinedione or derivative

thereof) promotes terminal differentiation of primary human liposarcoma cells. It has still further been discovered that RXR-specific ligands are also potent adipogenic agents in cells expressing the PPAR $\gamma$ /RXR $\alpha$  heterodimer, and that  
5 simultaneous treatment of liposarcoma cells with a thiazolidinedionyl moiety (a PPAR $\gamma$ -selective class of compounds) and an RXR-specific ligand results in an additive stimulation of differentiation. Accordingly, according to the invention, there have been identified  
10 compositions which are useful for the treatment of liposarcomas.

#### DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, there are provided methods for the treatment of liposarcomas,  
15 said method comprising administering to a subject in need of such treatment an amount of a therapeutic composition effective to ameliorate the effects of neoplastic cell proliferation of liposarcoma cells, wherein said therapeutic composition comprises at least one  
20 thiazolidinedione and at least one retinoid X receptor (RXR) selective agonist in a pharmaceutically acceptable carrier therefor. Invention methods can also be used in a prophylactic manner, i.e., to prevent the onset of liposarcoma.

25 Thiazolidinediones contemplated for use in the practice of the present invention can be described broadly with reference to the general structure I:



10 wherein:

each of  $X_1$ ,  $X_2$ ,  $X_3$ ,  $X_4$ ,  $X_5$  and  $X_6$  is independently carbon, nitrogen, oxygen or sulfur, with the proviso that at least three of the atoms forming the ring are carbon,

15 A is:

$-Y_n-(CR^mR^n)_m-Z$ ,  
 $-Y_n-(CR^mR^n)_m-O-(CR^mR^n)_m-Z$ , or  
 $-Y_n-(CR^mR^n)_m-N(R''')-(CR^mR^n)_m-Z$ ,

wherein:

20 Y is -O- or -S-,

n is 0 or 1,

each  $R^m$  is independently hydrogen, lower alkyl, substituted lower alkyl, hydroxy, lower alkoxy, thioalkyl, halogen, trifluoromethyl, cyano, nitro, amino, carboxyl, carbamate, sulfonyl or sulfonamide,

25

$R'''$  is hydrogen, lower alkyl or substituted alkyl,

30

m falls in the range of 1 up to 15,

each  $m'$  falls independently in the range of 1 up to 8,

each  $m''$  falls independently in the range of 0 up to 12, and

35

Z is a thiazolidinedionyl moiety;

$R_2$  is hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl,

alkylaryl, substituted alkylaryl,  
 alkenylaryl, substituted alkenylaryl,  
 alkynylaryl, substituted alkynylaryl,  
 arylalkyl, substituted arylalkyl,  
 arylalkenyl, substituted arylalkenyl,  
 arylalkynyl, substituted arylalkynyl,  
 oxyalkyl, poly(alkylene oxide), substituted  
 poly(alkylene oxide); poly(alkylene  
 sulfide), substituted poly(alkylene  
 sulfide), poly(alkylene amine) or  
 substituted poly(alkylene amine); with  $R_2$   
 having in the range of 1 up to about 15  
 carbon atoms being preferred;

$R_3$  is hydrogen, hydroxy, halogen, alkoxy, lower  
 alkyl, substituted lower alkyl, alkenyl,  
 substituted alkenyl, alkynyl or substituted  
 alkynyl; with  $R_3$  having in the range of 0 up  
 to about 6 carbon atoms being preferred;

$R_4$  is hydrogen, formyl, acyl, lower alkyl,  
 substituted lower alkyl or a  
 thiazolidinedionyl moiety; with  $R_4$  having in  
 the range of 0 up to about 4 carbon atoms  
 being preferred;

$R_5$  is hydrogen, hydroxy, lower alkoxy, lower  
 alkyl, substituted lower alkyl, alkenyl,  
 substituted alkenyl, alkynyl, substituted  
 alkynyl or halogen; with  $R_5$  having in the  
 range of 0 up to about 6 carbon atoms being  
 preferred; and

$R_6$  is hydrogen, hydroxy, lower alkoxy, lower  
 alkyl, substituted lower alkyl, alkenyl,  
 substituted alkenyl, alkynyl, substituted  
 alkynyl or halogen; with  $R_6$  having in the  
 range of 0 up to about 6 carbon atoms being  
 preferred.

Those of skill in the art recognize that the core ring of structure I can be any one of a number of different aromatic or pseudo-aromatic structures, e.g., a benzene ring, a pyridine ring, a pyrazine, an oxazine, and the like.

As employed herein, "lower alkyl" refers to straight or branched chain alkyl groups having in the range of about 1 up to 4 carbon atoms; "alkyl" refers to straight or branched chain alkyl groups having in the range of about 1 up to 12 carbon atoms; "substituted alkyl" refers to alkyl groups further bearing one or more substituents such as hydroxy, alkoxy (of a lower alkyl group), mercapto (of a lower alkyl group), halogen, trifluoromethyl, cyano, nitro, amino, carboxyl, carbamate, sulfonyl, sulfonamide, heteroatom-containing cyclic moieties, substituted heteroatom-containing cyclic moieties, and the like.

As employed herein, "alkenyl" refers to straight or branched chain hydrocarbyl groups having at least one carbon-carbon double bond, and having in the range of about 2 up to 12 carbon atoms and "substituted alkenyl" refers to alkenyl groups further bearing one or more substituents as set forth above.

As employed herein, "alkynyl" refers to straight or branched chain hydrocarbyl groups having at least one carbon-carbon triple bond, and having in the range of about 2 up to 12 carbon atoms, and "substituted alkynyl" refers to alkynyl groups further bearing one or more substituents as set forth above.

As employed herein, "aryl" refers to aromatic groups having in the range of 6 up to 14 carbon atoms and "substituted aryl" refers to aryl groups further bearing one or more substituents as set forth above.



As employed herein, "alkylaryl" refers to alkyl-substituted aryl groups and "substituted alkylaryl" refers to alkylaryl groups further bearing one or more substituents as set forth above.

5 As employed herein, "alkenylaryl" refers to alkenyl-substituted aryl groups and "substituted alkenylaryl" refers to alkenylaryl groups further bearing one or more substituents as set forth above.

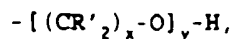
10 As employed herein, "alkynylaryl" refers to alkynyl-substituted aryl groups and "substituted alkynylaryl" refers to alkynylaryl groups further bearing one or more substituents as set forth above.

15 As employed herein, "arylalkyl" refers to aryl-substituted alkyl groups and "substituted arylalkyl" refers to arylalkyl groups further bearing one or more substituents as set forth above.

20 As employed herein, "arylalkenyl" refers to aryl-substituted alkenyl groups and "substituted arylalkenyl" refers to arylalkenyl groups further bearing one or more substituents as set forth above.

As employed herein, "arylalkynyl" refers to aryl-substituted alkynyl groups and "substituted arylalkynyl" refers to arylalkynyl groups further bearing one or more substituents as set forth above.

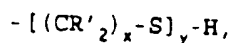
25 As employed herein, "poly(alkylene oxide)" refers to compounds having the general structure:



wherein each R' is independently selected from hydrogen or lower alkyl, x falls in the range of 1 up to about 4 and y

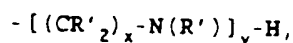
falls in the range of 2 up to about 8; "substituted poly(alkylene oxide)" refers to poly(alkylene oxide) groups further bearing one or more substituents as set forth above.

- 5 As employed herein, "poly(alkylene sulfide)" refers to compounds having the general structure:



- wherein R', x and y are as defined above; "substituted poly(alkylene sulfide)" refers to poly(alkylene sulfide) groups further bearing one or more substituents as set forth above.

As employed herein, "poly(alkylene amine)" refers to compounds having the general structure:

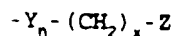


- 15 wherein R', x and y are as defined above; "substituted poly(alkylene amine)" refers to poly(alkylene amine) groups further bearing one or more substituents as set forth above.

20 As employed herein, "acyl" refers to alkyl-carbonyl species.

As employed herein, "halogen" or "halo" refers to fluoro substituents, chloro substituents, bromo substituents or iodo substituents.

25 In a presently preferred aspect of the present invention, "A" of Formula I has the structure:



wherein:

- Y is -O- or -S-,
- n is 0 or 1,
- x falls in the range of 2 up to 12; and
- 5 Z is a thiazolidinedionyl moiety.

In another preferred aspect of the present invention, "R<sub>2</sub>" of Formula I is methyl, ethyl, propyl, butyl, methoxy, ethoxy, propoxy, butoxy, and the like.

- 10 In yet another preferred aspect of the present invention, "R<sub>3</sub>" of Formula I is hydrogen, hydroxy, alkoxy, and the like.

In still another preferred aspect of the present invention, "R<sub>4</sub>" of Formula I is selected from formyl, acyl, a thiazolidenedionyl moiety, and the like.

- 15 In a further preferred aspect of the present invention, "R<sub>5</sub>" of Formula I is hydrogen.

In a still further preferred aspect of the present invention, "R<sub>6</sub>" of Formula I is hydrogen.

- 20 In yet another preferred aspect of the present invention, at least one of R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub>, R<sub>5</sub> and R<sub>6</sub> (in addition to A) is not hydrogen. It is especially preferred that at least two of R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub>, R<sub>5</sub> and R<sub>6</sub> (in addition to A) are not hydrogen. A plurality of substituents on the ring of structure I is especially preferred when m or the sum of
- 25 (m' + m"), with reference to the backbone of A, is less than or equal to 6.

Presently preferred species contemplated for use in the practice of the present invention include compounds wherein:

A is a thiazolidinedionyl moiety (e.g.,  
 $-O-(CH_2)_y$ -thiazolidenedione, wherein y falls  
 in the range of about 2 up to 8),

R<sub>2</sub> is hydrogen or lower alkyl,

5 R<sub>3</sub> is hydroxy or alkoxy,

R<sub>4</sub> is acyl or a thiazolidenedionyl moiety; and

R<sub>5</sub> and R<sub>6</sub> are each hydrogen.

The above-described compounds can be readily  
 prepared using a variety of synthetic methods, as are well  
 10 known by those of skill in the art. For example, many of  
 the above-described compounds can be prepared chemically or  
 enzymatically.

RXR selective ligands contemplated for use in the  
 practice of the present invention include substituted  
 15 benzoic acids or derivatives thereof (e.g., substituted  
 benzoates), substituted nicotinic acids or derivatives  
 thereof (e.g., substituted nicotines), substituted  
 carboxylated furans, and the like. Exemplary agonists  
 contemplated for use herein include 4-[1-(3,5,5,8,8-  
 20 pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoic  
 acid, 1,3-propylene glycol ketal of 4-[1-(5,5,8,8-  
 tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoic  
 acid, methyl 4-[(3,8,8-trimethyl-5,6,7,8-  
 tetrahydronaphthalen-2-yl)carbonyl]benzoate, methyl  
 25 4-[(3,5,5-trimethyl-5,6,7,8-tetrahydronaphthalen-2-  
 yl)carbonyl]benzoate, methyl 4-[(1,1,2,3,3,6-  
 hexamethylindan-5-yl)carbonyl]benzoate, methyl  
 6-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-  
 yl)carbonyl]nicotinate, methyl 4-[1-(3,8,8-trimethyl-  
 30 5,6,7,8-tetrahydro-2-naphthalen-2-yl)ethenyl]benzoate,  
 methyl 4-[1-(3,5,5-trimethyl-5,6,7,8-tetrahydronaphthalen-  
 2-yl)ethenyl]benzoate, methyl 4-[1-(1,1,2,3,3,6-  
 hexamethylindan-5-yl)ethenyl]benzoate, methyl 6-[1-  
 35 yl)ethenyl]nicotinate, 4-[1-(3,8,8-trimethyl-5,6,7,8-

tetrahydronaphthalen-2-yl)ethenyl]benzoic acid,  
 4-[1-(3,5,5-trimethyl-5,6,7,8-tetrahydro-2-naphthalen-2-yl)ethenyl]benzoic acid, 4-[1-(1,1,2,3,3,6-hexamethylindan-5-yl)ethenyl]benzoic acid, 6-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)ethenyl]nicotinic acid,  
 5 methyl 4-[1-methyl-1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)ethyl]benzoate, 4-[2-methyl-1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)ethyl]benzoic acid, 4-[1-methyl-1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)ethyl]benzoic acid, 4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)ethyl]benzoic acid, methyl 4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopropyl]benzoate, methyl 4-[2-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)oxiranyl]benzoate, methyl 6-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopropyl]nicotinate,  
 15 4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopropyl]benzoic acid, 4-[2-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)oxiranyl]benzoic acid, 6-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopropyl]nicotinic acid (also referred to in the art as "LG268"), 3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-ol, methyl 4-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)methyl]benzoate, methyl 4-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)oxy]benzoate, 4-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)methyl]benzoic acid, 4-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)oxy]benzoic acid, methyl 2-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)carbonyl]benzoate, methyl 3-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)carbonyl]benzoate, 2-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)carbonyl]benzoic acid, 3-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)carbonyl]benzoic acid,  
 30 the carboxylated furan derivative referred to as AGN191701

(see Mol. and Cell. Biol. 15:3540-3551 (1995)), and the like.

Presently preferred RXR selective agonists contemplated for use herein include 6-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopropyl]nicotinic acid and 4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)ethenyl]benzoic acid.

Combinations of thiazolidenediones with RXR selective agonists contemplated for use in the practice of the present invention can be employed for both in vitro and in vivo applications. For in vivo applications, the above-described compositions can be incorporated into a pharmaceutically acceptable formulation for administration. Those of skill in the art can readily determine suitable dosage levels when compositions contemplated for use in the practice of the present invention are so used.

In accordance with another embodiment of the present invention, there are provided compositions comprising at least one thiazolidenedione and at least one retinoid X receptor (RXR) selective agonist, wherein said thiazolidenedione has the structure I, as described hereinabove.

In accordance with a particular embodiment of the present invention, compositions comprising at least one thiazolidenedione, an agonist of RXR, and a pharmaceutically acceptable carrier are also contemplated. Exemplary pharmaceutically acceptable carriers include carriers suitable for oral, intravenous, subcutaneous, intramuscular, intracutaneous, and the like administration. Administration in the form of creams, lotions, tablets, dispersible powders, granules, syrups, elixirs, sterile

aqueous or non-aqueous solutions, suspensions or emulsions, and the like, is contemplated.

For the preparation of oral liquids, suitable carriers include emulsions, solutions, suspensions, syrups, and the like, optionally containing additives such as wetting agents, emulsifying and suspending agents, sweetening, flavoring and perfuming agents, and the like.

For the preparation of fluids for parenteral administration, suitable carriers include sterile aqueous or non-aqueous solutions, suspensions, or emulsions. Examples of non-aqueous solvents or vehicles are propylene glycol, polyethylene glycol, vegetable oils, such as olive oil and corn oil, gelatin, and injectable organic esters such as ethyl oleate. Such dosage forms may also contain adjuvants such as preserving, wetting, emulsifying, and dispersing agents. They may be sterilized, for example, by filtration through a bacteria-retaining filter, by incorporating sterilizing agents into the compositions, by irradiating the compositions, or by heating the compositions. They can also be manufactured in the form of sterile water, or some other sterile injectable medium immediately before use.

PPAR $\gamma$  is expressed at high levels in the adipose tissues of mouse and rat (see, for example, Tontonoz et al., in *Genes Dev.* 8:1224-1234 (1994) and Braissant et al., in *Endocrinology* 137:354-366(1996)). To determine the tissue distribution of this receptor in humans, Northern analysis of RNA prepared from a variety of human tissues was performed. Human PPAR $\gamma$  is seen to be expressed at highest levels in adipose tissue and at lower levels in several other tissues including lung and kidney. As a control, the blot was also hybridized with cDNA for the adipocyte-specific binding protein aP2. The heart and muscle samples can be seen to contain small amounts of aP2

mRNA, suggesting that these tissues also contain some adipose cells.

Tumorigenesis frequently involves the inactivation or downregulation of genes responsible for initiating and maintaining a differentiated phenotype. As PPAR $\gamma$  appears to play a central role in the adipocyte differentiation process, the expression of this receptor was examined in a series of human liposarcomas. This series included RNA prepared from each of the three major histologic subtypes of liposarcoma; well differentiated/dedifferentiated, myxoid/round cell and pleomorphic. The histologic and cytogenetic characteristics of each tumor is given in Table 3 (see Example 2). For the most part, the well differentiated/dedifferentiated tumors exhibited ring chromosomes and giant marker chromosomes, the myxoid/round cell liposarcomas exhibited the characteristic t(12;16)(13p11) translocation, and the pleomorphic forms exhibited complex rearrangements. Surprisingly, despite their block in differentiation, each liposarcoma examined was found to express high levels of PPAR $\gamma$  RNA, comparable to that of normal fat. These results suggest that most if not all liposarcomas have been transformed at a point in the differentiation process after induction of PPAR $\gamma$  expression. In contrast, PPAR $\gamma$  RNA was not expressed at significant levels in any other type of soft tissue sarcoma examined, including leiomyosarcoma (n=4), fibrosarcoma (n=1), angiosarcoma (n=1), malignant peripheral nerve sheath tumor (MPNS, n=1), or malignant fibrous histiocytoma (MFH, n=1). Thus, PPAR $\gamma$  may be a sensitive marker for distinguishing liposarcoma from other histologic types of soft tissue sarcoma.

Transient transfection experiments were performed to characterize the activation profile of human PPAR $\gamma$ . To eliminate interference from endogenous receptor in the



transfected cells, a chimeric PPAR $\gamma$  receptor was utilized that could activate transcription through a heterologous response element (see Forman et al., in Cell 83:803-812 (1995)). This chimeric protein contained the yeast GAL4 DNA binding domain linked to the ligand binding domain of human PPAR $\gamma$ . The GAL4-hPPAR $\gamma$  expression vector was cotransfected into CV-1 cells with a luciferase reporter plasmid containing the GAL4 upstream activating sequence. In this assay, the thiazolidinediones BRL49653, troglitazone and pioglitazone are all seen to be effective activators of human PPAR $\gamma$ .

Liposarcomas have presumably acquired one or more genetic defects that interfere with the course of normal adipocyte development. The observation that PPAR $\gamma$  is expressed consistently in these tumors raised the possibility that the malignant cells might be forced to complete the differentiation program by maximally activating the PPAR $\gamma$  pathway. To address this possibility, primary cells isolated from three human liposarcomas were cultured in vitro. Primary cell strains LS857 and LS175 were derived from well differentiated liposarcomas and LS707 was derived from an intermediate grade myxoid/round cell liposarcoma (see Table 3). High grade pleomorphic liposarcoma cells could not be expanded to sufficient numbers to permit studies of differentiation. A primary leiomyosarcoma cell line LM203 was cultured as a control. To confirm that these cultures consisted of malignant tumor-derived cells, cytogenetic analysis was performed. As shown in Table 3, the karyotype of the cells in each culture was characteristic of the parent liposarcoma.

When cultured in the presence of fetal bovine serum and insulin, conditions permissive for adipocyte differentiation, all three cell lines maintain a fibroblastic morphology. LS175 cells contained small amounts of stainable lipid under these conditions. When

cultures were treated for 7 days with 10  $\mu$ M of the PPAR $\gamma$  ligand pioglitazone, the cells readily accumulated lipid and adopted a morphology characteristic of mature culture adipocytes. No lipid accumulation was observed with the LM203 leiomyosarcoma cells, which do not express PPAR $\gamma$ . The degree of morphologically recognizable differentiation varied from 40% in the LS857 cells to 75% in the LS175 cells. After induction for 7 days with thiazolidinedione, cells maintained their differentiated morphology even when pioglitazone was withdrawn. This experiment was performed at least twice with each cell strain with quantitatively and qualitatively similar results. Induction of differentiation was also observed with the thiazolidinediones BRL49653 and troglitazone, while no effect was observed with compound 66, the inactive synthetic precursor to BRL49653.

Simultaneous exposure of competent cells to both PPAR $\gamma$  and RXR-specific ligand was investigated to determine if such combination might provide a stronger adipogenic signal than a PPAR $\gamma$  ligand alone. The ability of the RXR-specific ligand LG268 to promote adipocyte differentiation was investigated using NIH-3T3 fibroblasts that express PPAR $\gamma$  from a retroviral vector (see Tontonoz et al, in Cell 79:1147-1156 (1994)). It has previously been shown that wild-type NIH-3T3 cells express RXR $\alpha$  but not PPAR $\gamma$ . NIH-vector and NIH-PPAR $\gamma$  cells were cultured as described in the Examples. At confluence, cells were treated for 7 days with no activator, 1  $\mu$ M pioglitazone alone, 50 nM LG268 alone, or 5  $\mu$ M thiazolidinedione and 50 nM LG268. After an additional four days of culture, cells were fixed and stained with oil red O. Data are presented in Table 1 as the range of morphologically recognizable differentiation observed for each line over three separate experiments.

Table 1  
‡ lipid containing cells

<u>cell line</u>	<u>no activator</u>	<u>+pioglitazone</u>	<u>+LG268</u>	<u>+pioglitazone+LG268</u>
NIH	0	0	0	<1
5 NIH-PPAR $\gamma$	2-5	60-70	50-65	>90

As shown in Table 1, treatment of confluent NIH-PPAR $\gamma$  cells for 7 days with 50 nM LG268 resulted in significant stimulation of adipocyte differentiation, comparable to that seen with 7 days of treatment with 1  $\mu$ M pioglitazone alone. Thus simultaneous exposure to both activators resulted in an additive effect. LG268 had no effect on NIH-vector cells, indicating that the adipogenic activity of this compound, like that of pioglitazone, is dependent on the presence of PPAR $\gamma$ . Similar results are obtained with the preadipocyte cell lines 3T3-L1 and 3T3-F442A, which express both PPAR $\gamma$  and RXR $\alpha$ . Northern analysis confirms that pioglitazone and LG268 have an additive effect on the induction of the adipocyte-specific genes aP2 and adipsin in NIH-PPAR $\gamma$  cells. No induction of adipocyte gene expression was observed in NIH-vector cells under similar conditions.

The ability of LG268 to promote differentiation of human liposarcoma cells was then examined. Treatment of LS857 cells for 7 days with 50 nM LG268 led to a significant degree of adipocyte differentiation, similar to that seen with 10  $\mu$ M pioglitazone alone. When LS857 cells were treated simultaneously with LG268 and a thiazolidinedione (either pioglitazone or BRL449653), an additive effect on differentiation was observed. To further characterize the effects of PPAR $\gamma$  and RXR ligands on liposarcoma cells, the expression of adipocyte-specific markers were examined by Northern blotting. LS857 cells, like the tumor from which they were derived, express PPAR $\gamma$

mRNA. Treatment of LS857 cells with pioglitazone leads to the induction of two markers of terminal adipocyte differentiation, the MRNAs encoding aP2 and adipsin. Simultaneous treatment with pioglitazone and LG268 results in an additive induction of adipocyte gene expression. In summary, treatment of LS857 cells with thiazolidinediones and RXR-specific retinoids leads to changes in morphology and gene expression consistent with terminal adipocyte differentiation.

10           Terminal differentiation of white adipocytes in vitro and in vivo is characterized by permanent withdrawal from the cell cycle. A critical question is whether thiazolidinedione-induced differentiation of liposarcoma cells is accompanied by growth arrest. To address this issue, LS857 cells were cultured in the presence or absence of pioglitazone. Following induction of morphologic differentiation, pioglitazone was withdrawn. After 48 hours of continued culture in the absence of pioglitazone, cells were labeled for 48 hours with 5-bromo-2'-deoxyuridine (BrdU). Cells undergoing DNA synthesis during the labeling period should stain positive for BrdU incorporation after fixation and incubation with an enzyme-linked monoclonal antibody (see Examples).

25           LS857 cells were cultured in the presence or absence of pioglitazone. Following induction of morphologic differentiation, pioglitazone was withdrawn. After 48 hours of continued culture in the absence of pioglitazone, cells were labeled for 48 hours with bromodeoxyuridine, stained using an enzyme-linked monoclonal antibody (see Examples) and visualized microscopically. The number of cells staining positive for BrdU and/or containing visible cytoplasmic lipid is indicated in Table 2.

Table 2

Experiment	#cells counter	#BrdU+ (%)	#lipid + (%)	#lipid + /BrdU+ (%)
control	500	232(46)	0	NA
PIO/LG #1	510	173(34)	204(40)	22(4)
5 PIO/LG #2	595	156(26)	233(51)	17(3)

In the experiments shown in Table 2 (PIO/LG268#1 and PIO/LG268#2), 26-34% of the cells contained visible cytoplasmic lipid. 40-51% of the cells in this culture stained positive for BrdU incorporation by light microscopy; however, of those cells containing lipid, only 3-4% stain positive for BrdU. When differentiated cultures were trypsinized and replated, lipid-containing cells failed to reenter the cell cycle as determined by BrdU labeling. These results demonstrate the thiazolidinedione-induced differentiation of LS857 cells leads to cell cycle withdrawal.

Termination differentiation of most specialized cell types, including white adipocytes, is linked to cell cycle withdrawal. Tumorigenesis is characterized by a loss of cell cycle control and a concordant block in the differentiation program. It has been demonstrated herein that most human liposarcomas express high levels of the adipocyte regulatory complex PPAR $\gamma$ /RXR $\alpha$  and that PPAR $\gamma$  and RXR $\alpha$ -specific ligands are able to trigger terminal differentiation of primary human liposarcoma cells *in vitro*. These results suggest that the developmental defect in most liposarcomas is downstream of PPAR $\gamma$  expression, and that in at least some tumor cells this developmental block can be overcome by maximal activation of the PPAR $\gamma$  pathway.

While the precise nature of the developmental defects in liposarcoma is not yet clear, it is likely these defects ultimately lead to the inactivation or antagonism

of one or more adipocyte transcriptional regulatory proteins. Members of both the C/EBP and PPAR transcription factor families have been shown to play central complementary roles in adipogenesis in murine models (see, for example, Cornelius et al., in *Ann. Rev. Nutr.* **14**:771-774 (1994), Tontonoz et al., in *Curr. Opin. Genet. Dev.* **5**:571-576 (1995), Freytag et al., in *Genes Dev.* **8**:1654-1663 (1994), Wu et al., in *Genes Dev.* **9**:2350-2363 (1995) and Yeh et al., in *Genes Dev.* **9**:168-181 (1995)). Interestingly, the C/EBP family has previously been implicated in the pathogenesis of human myxoid liposarcoma through the characterization of the t(12:16) translocation associated with this tumor. This rearrangement fuses the gene for the C/EBP family member CHOP on chromosome 12 to that of the RNA binding protein TLS on chromosome 16 (see Crozat et al., in *Nature* **363**:640-644 (1993)). CHOP lacks a transcriptional activation domain and has therefore been postulated to function as a dominant negative regulator of other C/EBP proteins. The precise mechanism whereby TLS/CHOP contributes to differentiation arrest and tumorigenesis, however, remains to be elucidated.

The mechanisms by which differentiation is coupled to cessation of cell growth are not fully understood; however, there is mounting evidence that key proteins controlling differentiation interact directly with the cell cycle machinery. For example, the myogenic transcription factor MyoD has been shown to be negatively regulated by cyclin D1-dependent kinase and to induce expression of the cdk inhibitor p21 (see, for example, Halevy et al., in *Science* **267**:1018-1021 (1995) and Skapek et al., in *Science* **267**:1022-1024 (1995)).

The impact of RXR-specific activators on adipocyte differentiation has not previously been addressed. It is demonstrated herein that RXR-specific retinoids can function as adipogenic regulators through

activation of the PPAR $\gamma$ /RXR $\alpha$  heterodimer, and that the adipogenic activity of the heterodimer is maximal when both receptors are bound by their respective ligands. Given that PPAR $\gamma$  is likely to be the biologic receptor mediating the insulin-sensitizing effects of the thiazolidinediones, this observation suggests that RXR-specific ligands may also have insulin-sensitizing activity in vivo. Moreover, the insulin-sensitizing effects of thiazolidinedione ligands for PPAR $\gamma$  might be enhanced by simultaneous administration of an RXR-specific ligand.

The results presented herein have important implications for the pharmacologic management of liposarcoma in humans. Liposarcoma is currently managed with surgery and a judicious combination of chemotherapy and radiotherapy. Despite conventional multimodality therapy, anywhere from 25 to 75% of patients with advanced liposarcoma will die from their disease within 5 years. The present results suggest that a combination of the thiazolidinedione class of antidiabetic drugs and RXR-specific retinoids may be useful as a non-toxic alternative to conventional chemotherapy for the treatment of disseminated or locally advanced liposarcoma. Members of the thiazolidinedione class of drugs have undergone extensive preclinical testing as anti-diabetic agents. Troglitazone is currently in phase three clinical trials in the U.S. and studies have supported its usefulness in NIDDM (see Nolan et al., in N. Engl. J. Med. 331:1188-1193 (1994)). Other thiazolidinediones have been approved for clinical use in Japan. Although certain thiazolidinediones have been associated with some degree of toxicity in long-term use as insulin sensitizing agents, this should not preclude their use as antineoplastic agents as conventional chemotherapy is associated with far greater toxicity. The ability of a combination of thiazolidinediones and RXR-specific retinoids to induce differentiation of liposarcoma cells in vitro strongly suggests that these compounds may

also be able to stimulate differentiation and growth arrest of human tumors in vivo.

The invention will now be described in greater detail by reference to the following non-limiting examples.

5

#### Example 1

##### Tissue samples and cytogenetics

Normal human tissues, liposarcomas and other soft tissue sarcomas were obtained from surgical cases at the Brigham and Women's Hospital, Boston. All tissue samples were taken from homogeneous and viable portions of the resected sample by the pathologist and frozen within 10 minutes of excision. Hematoxylin and eosin stained sections of each soft tissue sarcoma were reviewed by a single pathologist (C.F.) and classified according to histologic type, grade, mitotic activity and surgical margin. Histologic classification was based solely on morphologic patterns recognition using conventional diagnostic criteria. Mitotic activity counts were performed with high power field size of  $0.120\text{mm}^2$  and at least 50 high power fields were counted from the most cellular areas of the tumor. for cytogenetic analysis tumors were disaggregated with collagenase and harvested after 3-7 days of culture in T25 flasks (see Fletcher et al., in N. Eng. J. Med. 324:436-443 (1991)). Metaphase cells were analyzed by trypsin-Giemsa (see Seabright in Lancet 2:971-972 (1971)) and quinacrine mustard banding (see Fletcher et al., in Am. J. Pathol. 138:515 (1991)).

#### Example 2

##### Northern analysis

30

Total RNA was prepared from tumors and normal human tissues by guanidium isothiocyanate extraction and CsCl centrifugation. RNA was electrophoresed through



formaldehyde-agarose gels, blotted to BioTrans nylon membranes (ICN) and hybridized as directed by the manufacturer. cDNA probes were labeled with [ $\alpha$ - $^{32}$ P]-dCTP by the random priming method to a specific activity of at least  $10^9$  cpm/ $\mu$ g.

To determine expression of PPAR $\gamma$  mRNA in human liposarcomas and other soft tissue sarcomas, total TNA (15  $\mu$ g per lane) was isolated from human tumors, electrophoresed through a formaldehyde-containing agarose gel, blotted to nylon and hybridized with  $^{32}$ P-labeled hPPAR $\gamma$  cDNA as described above. Equivalent amounts of intact RNA were run in each lane as indicated by ethidium bromide staining of the membrane after transfer and hybridization to a 36B4 cDNA probe. The histologic and cytogenetic characteristics of each tumor analyzed are summarized in Table 3.

TABLE 3

	<u>Tumor</u>	<u>Histology</u>	<u>Cytogenetics</u>	<u>Mitotic Index</u>	<u>Cell Culture</u>
20	107SP	well differentiated liposarcoma	47-48,XX, + 1-2mars	0.2	NA
	115SP	high grade myxoid/round cell liposarcoma	80-91,XXXX, t(12;16)(q13;p11)x2	6.0	NA
	116SP	high grade liposarcoma with pleomorphic, myxoid, well differentiated areas	ND	19.8	NA
	200SP	high grade liposarcoma, mixed pleomorphic and round cell	ND	7.2	NA
	203SP	well differentiated liposarcoma	48-50,XY,del(16)(q36), + 2-4 r	ND	LS175
25	204SP	well differentiated liposarcoma	48,XX,add(7)(136), del(11)(p13), + 2 mars	ND	LS857
	P144	well differentiated liposarcoma	48,XX, + 2r	0	NA

P147	well differentiated liposarcoma lipoma-like, sclerosing	46-49,XX,add(9)(q34), +1-2r, +1-2mars	0	NA
P154	atypical lipoma/well differentiated liposarcoma	ND	0	NA
P155	intermediate grade liposarcoma myxoid > round cell component	46,XY,t(12;16)(q13;p11)	1.0	LS707
P156	intermediate grade liposarcoma round cell > myxoid component	49XY, +del(1)(p32), +2,+8,t(12;16)(q13;p11)	1.0	NA
5 P158	well differentiated liposarcoma	ND	0	NA
P160	well differentiated liposarcoma with dedifferentiated areas	43-49,XX,add(1)(q43), -11,-13,-13,+1-3r	1.1	NA

PPAR $\gamma$  is observed to be expressed in multiple histologic types of human liposarcoma, while PPAR $\gamma$  is not expressed in other human soft tissue sarcomas.

### Example 3

#### Cell culture

Primary liposarcoma cells were isolated from selected freshly harvested tumors as described previously (see Sreekantaiah et al., in Am. J. Pathol. 144:1121-1134 (1994) and Fletcher et al., in Am. J. Pathol. 138:515 (1991), as well as references cited therein. Primary cells were plated at a density of at least  $2 \times 10^5$  cells/ml and cultured in 60 mm dishes in RPMI containing 15% Cosmic Calf Serum (Hyclone), 20  $\mu$ g/ml bovine pituitary extract (Collaborative Research) and 5  $\mu$ g/ml insulin.

PPAR $\gamma$ - and RXR-specific ligands induce expression of markers of terminal adipocyte differentiation in PPAR $\gamma$ -expressing fibroblasts and human liposarcoma cells. NIH-vector, NIH-PPAR $\gamma$  and LS857 cells were cultured as

described above. At confluence cells were treated for 7 days with no activator, pioglitazone alone, LG268 alone, or pioglitazone and LG268 as indicated. Total RNA (10  $\mu$ g per lane) was isolated from, electrophoresed through a formaldehyde-containing agarose gels, blotted to nylon and hybridized with  $^{32}$ P-labeled human or murine PPAR $\gamma$ , human or murine aP2, and human or murine adipsin cDNA using standard techniques. Equivalent amounts of intact RNA was run in each lane as indicated by ethidium bromide staining of the membrane after transfer and hybridization to a 36B4 cDNA probe.

In order to evaluate the induction of differentiation in human liposarcoma cells by thiazolidinediones and RXR-specific retinoids, LS857 cells were cultured as described above. At confluence, cells were treated for 7 days with no activator, 5  $\mu$ M thiazolidinedione (BRL 49653) or pioglitazone, 50 nM LG268, or 5  $\mu$ M thiazolidinedione and 50 nM LG268 as indicated. After an additional four days of culture, cells were fixed and stained with oil red O. Macroscopic view of the 60 mm culture dishes shows that thiazolidinedione alone or LG268 alone stimulate significant lipid accumulation, while simultaneous addition of both thiazolidinedione and LG268 has at least an additive effect on lipid accumulation, stimulating substantially higher levels of lipid accumulation than either agent alone.

#### Example 4

##### Transfection assays

The GAL4-hPPAR $\gamma$  expression vector was generously provided by V.K.K. Chatterjee. This construct contains residues 1-147 of GAL4 fused to residues 173-475 of hPPAR $\gamma$  and is driven by the SV40 early promoter (see Greene et al., in Gene Express. 4:281-299 (1995)). CV-1 cells were cultured in DMEM containing 10% resin-charcoal-stripped

- calf serum. Transfections were performed in phenol free DMEM containing 10% resin-charcoal-stripped fetal calf serum by the lipofection method using DOTAP (Boehringer Mannheim) according to the manufacturer's instructions.
- 5 After 2 hours, liposomes were removed and cells were cultured for an additional 40 hours in the presence or absence of thiazolidinediones as indicated. Luciferase and B-galactosidase assays were carried out as described previously (see Forman et al., in Cell 83:803-812 (1995)).
- 10 An SV40-GAL4/hPPAR $\gamma$  expression vector (100 ng/10<sup>5</sup> cells) was cotransfected into CV-1 cells with a UAS<sub>X4</sub> TK-LUC reporter plasmid (300 ng/0<sub>5</sub> cells) and a CMX-BGAL internal control plasmid (500 ng/10<sup>5</sup> cells) as described above. Following transfection cells were treated with
- 15 indicated concentrations of Pioglitazone (Upjohn), troglitazone (Parke Davis-Warner Lambert), BRL49653 (BIOMOL) and LG268 (Ligand Pharmaceuticals) were dissolved in DMSO and applied to cells in a volume of less than 5  $\mu$ l. The NIH-PPAR $\gamma$  and NIH-vector cells were derived by
- 20 retroviral infection as previously described (see Tontonoz et al., in Cell 79:1147-1156 (1994)) and cultured in DMEM containing 10% Cosmic Calf Serum and 5  $\mu$ g/ml insulin. Differentiated cells were fixed and stained for neutral lipid with oil red O. BrdU labeling was performed using
- 25 the 5-bromo-2'-deoxyuridine Labeling and Detection Kit II (Boehringer Mannheim) according to the manufacturer's instructions.

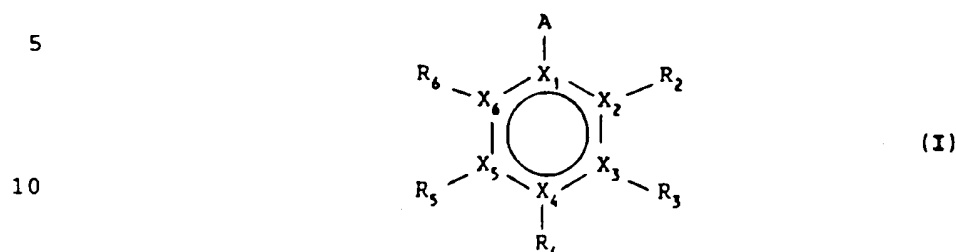
These experiments demonstrate that thiazolidinediones activate PPAR $\gamma$ s.

- 30 While the invention has been described in detail with reference to certain preferred embodiments thereof, it will be understood that modifications and variations are within the spirit and scope of that which is described and claimed..

That which is claimed is:

1. A method of treating liposarcomas, said method comprising administering to a subject in need of such treatment an amount of a therapeutic composition effective to ameliorate the effects of neoplastic cell proliferation of liposarcoma cells, wherein said therapeutic composition comprises at least one thiazolidinedione and at least one retinoid X receptor (RXR) selective agonist in a pharmaceutically acceptable carrier therefor.

2. A method according to claim 1 wherein said thiazolidinedione has the structure I, wherein structure I is as follows:



wherein:

15 each of  $X_1$ ,  $X_2$ ,  $X_3$ ,  $X_4$ ,  $X_5$  and  $X_6$  is independently carbon, nitrogen, oxygen or sulfur, with the proviso that at least three of the atoms forming the ring are carbon,

A is:

20  $-Y_n-(CR^*R^*)_n-Z$ ,  
 $-Y_n-(CR^*R^*)_n-O-(CR^*R^*)_m-Z$ , or  
 $-Y_n-(CR^*R^*)_n-N(R''')-(CR^*R^*)_m-Z$ ,

wherein:

Y is -O- or -S-,

n is 0 or 1,

25

each R" is independently hydrogen, lower alkyl, substituted lower alkyl, hydroxy, lower alkoxy, thioalkyl, halogen, trifluoromethyl, cyano, nitro, amino, carboxyl, carbamate, sulfonyl or sulfonamide,

30

R''' is hydrogen, lower alkyl or substituted alkyl,

35

m falls in the range of 1 up to 15,  
each m' falls independently in the range of 1 up to 8,

each m" falls independently in the range of 0 up to 12, and

40

Z is a thiazolidinedionyl moiety;  
R<sub>2</sub> is hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, alkylaryl, substituted alkylaryl, alkenylaryl, substituted alkenylaryl, alkynylaryl, substituted alkynylaryl, arylalkyl, substituted arylalkyl, arylalkenyl, substituted arylalkenyl, arylalkynyl, substituted arylalkynyl, oxyalkyl, poly(alkylene oxide), substituted poly(alkylene oxide), poly(alkylene sulfide), substituted poly(alkylene sulfide), poly(alkylene amine) or substituted poly(alkylene amine);

50

55

R<sub>3</sub> is hydrogen, hydroxy, halogen, alkoxy, lower alkyl, substituted lower alkyl, alkenyl, substituted alkenyl, alkynyl or substituted alkynyl;

60

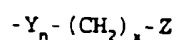
R<sub>4</sub> is hydrogen, formyl, acyl, lower alkyl, substituted lower alkyl or a thiazolidinedionyl moiety;

R<sub>5</sub> is hydrogen, hydroxy, lower alkoxy, lower alkyl, substituted lower alkyl, alkenyl,

substituted alkenyl, alkynyl, substituted alkynyl or halogen; and

65  $R_6$  is hydrogen, hydroxy, lower alkoxy, lower alkyl, substituted lower alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl or halogen.

3. A method according to claim 2 wherein A of Formula I has the structure:



wherein:

5 Y is -O- or -S-,  
 n is 0 or 1,  
 x falls in the range of 2 up to 12; and  
 Z is a thiazolidinedionyl moiety.

4. A method according to claim 1 wherein the substituents of Formula I are defined as follows:

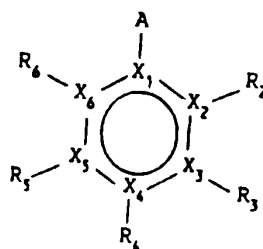
$R_2$  is methyl, ethyl, propyl, butyl, methoxy, ethoxy, propoxy or butoxy;  
 5  $R_3$  is hydrogen, hydroxy or alkoxy;  
 $R_4$  is formyl, acyl or a thiazolidenedionyl moiety;  
 $R_5$  is hydrogen; and  
 $R_6$  is hydrogen.

5. A method according to claim 2 wherein at least one of  $R_2$ ,  $R_3$ ,  $R_4$ ,  $R_5$  and  $R_6$  is not hydrogen.

6. A method according to claim 2 wherein at least two of  $R_2$ ,  $R_3$ ,  $R_4$ ,  $R_5$  and  $R_6$  are not hydrogen.

7. A method according to claim 6 wherein m or the sum of ( $m' + m''$ ), with reference to the backbone of A, is less than or equal to 6.

8. A method according to claim 2 wherein:  
 A is  $-O-(CH_2)_y$ -thiazolidinedionyl, wherein y falls  
 in the range of about 2 up to 8;  
 $R_2$  is hydrogen or lower alkyl,  
 $R_3$  is hydroxy or alkoxy,  
 $R_4$  is formyl, acyl or a thiazolidenedionyl  
 moiety; and  
 $R_5$  and  $R_6$  are each hydrogen.
9. A method according to claim 1 wherein said  
 retinoid X receptor (RXR) selective agonist is a  
 substituted benzoic acid or derivative thereof, a  
 substituted nicotinic acid or derivative thereof, or a  
 substituted carboxylated furan.
10. A method according to claim 9 wherein said  
 retinoid X receptor (RXR) selective agonist is  
 6-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-  
 yl)cyclopropyl]nicotinic acid (LG268).
11. A method according to claim 9 wherein said  
 retinoid X receptor (RXR) selective agonist is  
 4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-  
 yl)ethenyl]benzoic acid.
12. A composition comprising at least one  
 thiazolidenedione and at least one retinoid X receptor  
 (RXR) selective agonist, wherein said thiazolidenedione has  
 the structure I, wherein structure I is as follows:



(I)



wherein:

- 15 each of  $X_1$ ,  $X_2$ ,  $X_3$ ,  $X_4$ ,  $X_5$  and  $X_6$  is independently carbon, nitrogen, oxygen or sulfur, with the proviso that at least three of the atoms forming the ring are carbon,

A is:

- 20  $-Y_n-(CR^"R^")_m-Z$ ,  
 $-Y_n-(CR^"R^")_{m'}-O-(CR^"R^")_{m''}-Z$ , or  
 $-Y_n-(CR^"R^")_{m'}-N(R^{'''})-(CR^"R^")_{m''}-Z$ ,

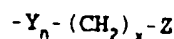
wherein:

- Y is -O- or -S-,  
 25 n is 0 or 1,  
 each  $R^"$  is independently hydrogen, lower alkyl, substituted lower alkyl, hydroxy, lower alkoxy, thioalkyl, halogen, trifluoromethyl, cyano, nitro,  
 30 amino, carboxyl, carbamate, sulfonyl or sulfonamide,  
 $R^{'''}$  is hydrogen, lower alkyl or substituted alkyl,  
 m falls in the range of 1 up to 15,  
 35 each  $m'$  falls independently in the range of 1 up to 8,  
 each  $m''$  falls independently in the range of 0 up to 12, and  
 Z is a thiazolidenedionyl moiety;  
 40  $R_2$  is hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, alkylaryl, substituted alkylaryl,  
 alkenylaryl, substituted alkenylaryl,  
 45 alkynylaryl, substituted alkynylaryl, arylalkyl, substituted arylalkyl, arylalkenyl, substituted arylalkenyl, arylalkynyl, substituted arylalkynyl, oxyalkyl, poly(alkylene oxide), substituted poly(alkylene oxide), poly(alkylene

50

- sulfide), substituted poly(alkylene sulfide), poly(alkylene amine) or substituted poly(alkylene amine);
- 55  $R_3$  is hydrogen, hydroxy, halogen, alkoxy, lower alkyl, substituted lower alkyl, alkenyl, substituted alkenyl, alkynyl or substituted alkynyl;
- $R_4$  is hydrogen, formyl, acyl, lower alkyl or substituted lower alkyl;
- 60  $R_5$  is hydrogen, hydroxy, lower alkoxy, lower alkyl, substituted lower alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl or halogen; and
- 65  $R_6$  is hydrogen, hydroxy, lower alkoxy, lower alkyl, substituted lower alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl or halogen.

13. A composition according to claim 12 wherein A of Formula I has the structure:



wherein:

- 5 Y is -O- or -S-,  
 n is 0 or 1,  
 x falls in the range of 2 up to 12; and  
 Z is a thiazolidinedionyl moiety.

14. A composition according to claim 12 wherein the substituents of Formula I are defined as follows:

- $R_2$  is methyl, ethyl, propyl, butyl, methoxy, ethoxy, propoxy or butoxy;  
 $R_3$  is hydrogen, hydroxy or alkoxy;  
 $R_4$  is formyl, acyl or a thiazolidenedionyl moiety;  
 $R_5$  is hydrogen; and

$R_6$  is hydrogen.

15. A composition according to claim 12 wherein at least one of  $R_2$ ,  $R_3$ ,  $R_4$ ,  $R_5$  and  $R_6$  is not hydrogen.

16. A composition according to claim 12 wherein at least two of  $R_2$ ,  $R_3$ ,  $R_4$ ,  $R_5$  and  $R_6$  are not hydrogen.

17. A composition according to claim 16 wherein  $m$  or the sum of ( $m' + m''$ ), with reference to the backbone of A, is less than or equal to 6.

18. A composition according to claim 12 wherein:  
A is  $-O-(CH_2)_y$ -thiazolidinedionyl, wherein  $y$  falls  
in the range of about 2 up to 8;

$R_2$  is hydrogen or lower alkyl,

5  $R_3$  is hydroxy or alkoxy,

$R_4$  is formyl, acyl or a thiazolidinedionyl moiety; and

$R_5$  and  $R_6$  are each hydrogen.

19. A composition according to claim 12 wherein said retinoid X receptor (RXR) selective agonist is a substituted benzoic acid or derivative thereof, a substituted nicotinic acid or derivative thereof, or a  
5 substituted carboxylated furan.

20. A composition according to claim 19 wherein said retinoid X receptor (RXR) selective agonist is 6-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)cyclopropyl]nicotinic acid (LG268).

21. A composition according to claim 19 wherein said retinoid X receptor (RXR) selective agonist is 4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)ethenyl]benzoic acid.



## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US97/24187

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 31/44, 31/425, 31/19

US CL : 514/356, 369, 569

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/356, 369, 569

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS AND CAS ONLINE: liposarcoma? with thiazolidinedione?, retinoid?

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y, P	Chem. abstr., Vol.126, 1997, (Columbus, OH, USA) the abstract No. 139558, TONTONOV, P. "Terminal Differentiation of Human Liposarcoma Cells Induced by Ligands for Peroxisome Proliferator-Activated Receptor $\gamma$ and the Retinoid X Receptor." Proc. Natl. Acad. Sci. U.S.A. 1997, 94(1), 237-241, see entire document.	1-21

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

Special categories of cited documents:	
*A* document defining the general state of the art which is not considered to be of particular relevance	*T* later documents published after the international filing date or priority date and not in conflict with the application but aimed to understand the principle or theory underlying the invention
*B* earlier document published on or after the international filing date	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*L* document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*O* document referring to an oral disclosure, use, exhibition or other means	*A* document member of the same patent family
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

12 MARCH 1998

Date of mailing of the international search report

10 APR 1998

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorizing Officer

KIMBERLY JORDAN

Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)\*

